Mixture Interpretation using Probabilistic Genotyping

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Statistical Analysis of DNA Typing Results

SWGDAM Guidelines 4.1. The laboratory must perform statistical analysis in support of any inclusion that is determined to be relevant in the context of a case, irrespective of the number of alleles detected and the quantitative value of the statistical analysis.

Buckleton & Curran (2008): “There is a considerable aura to DNA evidence. Because of this aura it is vital that weak evidence is correctly represented as weak or not presented at all.”

Statistical Approaches with Mixtures

See Ladd et al. (2001) Croat Med J. 42:244-246

<table>
<thead>
<tr>
<th>“Exclusionary” Approach</th>
<th>“Inferred Genotype” Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random Man Not Excluded (RMNE)</td>
<td>Random Match Probability (RMP)</td>
</tr>
<tr>
<td>Combined Prob. of Inclusion (CPI)</td>
<td>Combined Prob. of Exclusion (CPE)</td>
</tr>
<tr>
<td>Likelihood Ratio (LR)</td>
<td>Random Match Probability (mRMP)</td>
</tr>
</tbody>
</table>

**Statistical Approaches with Mixtures**

- **Random Man Not Excluded (CPE/CPI)** - The probability that a random person (unrelated individual) would be included/excluded as a contributor to the observed DNA mixture.

\[
CPI = (f(a) + f(b) + f(c) + f(d))^2
\]

\[
CPI = PI_{M1} \times PI_{M2} \cdots
\]

\[
CPE = 1 - CPI
\]

**“Advantages and Disadvantages”**

**RMNE (CPE/CPI)**

- **Advantages**
  - Does not require an assumption of the number of contributors to a mixture
  - Easier to explain in court
  - Deconvolution is not necessary

- **Disadvantages**
  - Weaker use of the available information (robs the evidence of its true probative power because this approach does not consider the suspect’s genotype).
  - Alleles below ST cannot be used for statistical purpose
  - There is a potential to include a non-contributor

Summarized from John Buckleton, Forensic DNA Evidence Interpretation, p. 223
Curran and Buckleton (2010)

(1) Created 1,000 2 person mixtures

\[ \text{e.g. vWA 12, 15, 13, 16} = 12, 13, 15, 16 \]

(2) Created 10,000 Random genotypes

(3) Compared "random person" to mixture data, calculated PI for included loci, ignored discordant alleles.

"the risk of producing apparently strong evidence against an innocent suspect by this approach was not negligible."

30% of the cases had a CPI < 0.01
48% of the cases had a CPI < 0.05

"It is false to think that omitting a locus is conservative as this is only true if the locus does not have some exclusionary weight."
Statistical Approaches with Mixtures

- **Random Match Probability (RMP)** – The major and minor components can be successfully separated into individual profiles. A random match probability is calculated on the evidence as if the component was from a single source sample.

\[
\text{RMP}_{\text{major}} = 2pq = 2 \times f(a) \times f(d)
\]

**ISFG Recommendations on Mixture Interpretation**

1. The likelihood ratio (LR) is the preferred statistical method for mixtures over RMNE
2. Scientists should be trained in and use LRs
3. Methods to calculate LRs of mixtures are cited
4. Follow Clayton et al. (1998) guidelines when deducing component genotypes
5. Prosecution determines \( H_p \) and defense determines \( H_d \); multiple propositions may be evaluated
6. When minor alleles are the same size as stutters of major alleles, then they are indistinguishable
7. Allele dropout to explain evidence can only be used with low signal DNA data
8. No statistical interpretation should be performed on alleles below threshold
9. Stochastic effects limit usefulness of heterozygote balance and mixture proportion estimates with low level DNA


**Likelihood Ratio**
Likelihood Ratios in Forensic DNA Work

- We evaluate the evidence \( E \) relative to alternative pairs of hypotheses.

- Usually these hypotheses are formulated as follows:
  - The probability of the evidence if the crime stain originated with the suspect or \( \Pr(E|S) \).
  - The probability of the evidence if the crime stain originated from an unknown, unrelated individual or \( \Pr(E|U) \).

\[
LR = \frac{\Pr(E|S)}{\Pr(E|U)}
\]

Statistical Approaches with Mixtures

- **Likelihood Ratio** - Comparing the probability of observing the mixture data under two (or more) alternative hypotheses; in its simplest form \( LR = 1/RMP \).

\[
\frac{P(E|H_1)}{P(E|H_2)} = \frac{1}{P(E|H_2)} = \frac{1}{2pq} = 1/RMP
\]

Challenging Mixtures - Uncertainty

- **If allele dropout is a possibility** (e.g., in a partial profile), then there is uncertainty in whether or not an allele is present in the sample...and therefore what genotype combinations are possible.

- **If different allele combinations are possible** in a mixture, then there is uncertainty in the genotype combinations that are possible...
Handling Complex Mixtures

- Stochastic thresholds are necessary in combination with CPI statistics
  - but a stochastic threshold may not hold much meaning for >2 person mixtures (due to potential allele sharing)

- Most labs are not adequately equipped to cope with complex mixtures
  - Extrapolating validation studies from simple mixtures will not be enough to create appropriate interpretation SOPs

David Balding (UK professor of statistical genetics): “LTDNA cases are coming to court with limited abilities for sound interpretation.” (Rome, April 2012 meeting)

Challenging Mixtures

<table>
<thead>
<tr>
<th>Allele</th>
<th>RFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>56</td>
</tr>
<tr>
<td>13</td>
<td>60</td>
</tr>
<tr>
<td>“Q”</td>
<td>??</td>
</tr>
</tbody>
</table>

What should we do with data below our Stochastic Threshold?

- Continue to use RMNE (CPI, CPE)
What should we do with data below our Stochastic Threshold?

- Continue to use RMNE (CPI, CPE) (not optimal)
- Use the Binary LR with 2p (not optimal)

\[
LR = \frac{1}{2pq} \quad LR = \frac{0}{2pq} \quad LR = \frac{?}{2pq}
\]

The Binary LR approach “2p”
The “2p” Rule

Stain = AA
Suspect = AA
LR = 5
LR = 100
f(a) = 0.10 \(1/p^2 = 100\) \(1/2p = 5\)

The “2p” Rule

Stain = AA
Suspect = AB
LR = 5
Exclusion
f(a) = 0.10 \(1/2p = 5\)

Whatever way uncertainty is approached, probability is the only sound way to think about it.

-Dennis Lindley
Probabilistic Approaches

- “Semi-Continuous” or “Fully Continuous”
  - Semi-Continuous – information is determined from the alleles present – peak heights are not considered.
  - Fully Continuous – incorporation of biological parameters (PHR [Hb], Mx ratio, Stutter percentage, etc…).

What should we do with data below our Stochastic Threshold?

- Continue to use RMNE (CPI, CPE) (not optimal)
- Use the Binary LR with 2p (not optimal)
- Semi-continuous methods with a LR (Drop models)

R. v Garside and Bates

- James Garside was accused of hiring Richard Bates to kill his estranged wife, Marilyn Garside.
- Marilyn was visiting her mother when someone knocked on the door. Marilyn answered and was stabbed to death.
- A profile from the crime scene stain gave a low-level DNA profile of the perpetrator.
Summary

<table>
<thead>
<tr>
<th>Locus</th>
<th>Mrs Gangide</th>
<th>Bates</th>
<th>CSP: minor component</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3</td>
<td>16,16</td>
<td>13,16</td>
<td>13</td>
</tr>
<tr>
<td>VWA</td>
<td>15,17</td>
<td>16,16</td>
<td>16</td>
</tr>
<tr>
<td>D16</td>
<td>11,12</td>
<td>11,12</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>20,20</td>
<td>19,22</td>
<td></td>
</tr>
<tr>
<td>D8</td>
<td>12,13</td>
<td>8,13</td>
<td>8</td>
</tr>
<tr>
<td>D21</td>
<td>30,32,2</td>
<td>30,31,2</td>
<td>31,2</td>
</tr>
<tr>
<td>D18</td>
<td>14,14</td>
<td>12,15</td>
<td>15</td>
</tr>
<tr>
<td>D19</td>
<td>12,14</td>
<td>12,15</td>
<td>15</td>
</tr>
<tr>
<td>TH01</td>
<td>9,3,9,3</td>
<td>7,7</td>
<td>7</td>
</tr>
<tr>
<td>FGA</td>
<td>23,25</td>
<td>21,21</td>
<td>21</td>
</tr>
</tbody>
</table>

Three alleles from Bates were not present in the evidence.

Court case

- The Crown expert dropped the D18 locus (gave a LR = 1) from the statistical results and used “2p” for D2 to give an overall odds for Bates of 1 in 610,000.
- David Balding argued for the defense that dropping loci is not conservative.

Balding and Buckleton (2009)

Interpreting low template DNA profiles

Present the “Drop model” for interpreting LT-DNA profiles
**Drop Model**

\[ P(E \mid H_2) = \frac{\Pr(\text{no Drop-out at 22}) \cdot \Pr(\text{Drop-out at 19}) \cdot \Pr(\text{Drop-in})}{\Pr(\text{Drop-out at 22}) \cdot \Pr(\text{Drop-in at 19}) \cdot \Pr(\text{No Drop-in})} \]

\[ = \frac{0.95 \cdot 0.05 \cdot 0.99}{0.05 \cdot 0.01} = 0.047 \]

The defense can now argue that someone else in the population unrelated to Bates was the true perpetrator!

**Drop Model**

\[ P(E \mid H_2) = \frac{\Pr(\text{Drop-out at 17}) \cdot \Pr(\text{Drop-out at 23}) \cdot \Pr(\text{Drop-in at 22})}{\Pr(\text{Drop-out at 17}) \cdot \Pr(\text{Drop-out at 23}) \cdot \Pr(\text{Drop-in at 22})} \]

\[ = \frac{0.05 \cdot 0.05 \cdot 0.01}{0.05 \cdot 0.05} = 0.000000675 \]

\[ = 0.0000025 \times 2pq_{17,23}(0.027) = 0.0000000675 \]
**Summary**

- Using “2p” for D2 gave a **LR = 11**. This is non-conservative compared to the probabilistic approach where a Pr(D) was incorporated into the calculation, the **LR = 2.8**

- The use of a probabilistic approach uses all of the information in the profile.

- The final LR in favor of the Hp was ≈ 400,000.

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**Software will help with the math...**

- **likeLTD** (likelihoods for low-template DNA profiles)

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**Some Semi-Continuous Examples**

- LR mix (Haned and Gill)
- Balding (likeLTD - R program)
- FST (NYOCME, Mitchell et al.)
- Kelly et al. (University of Auckland, ESR)
- Lab Retriever (Lohmueller, Rudin and Inman)
- Armed Expert (NicheVision)
- Puch-Solis et al. (LiRa and LiRaHT)
- GenoProof Mixture (Qualitype)
LR Mix

![LR Mix interface](image1)

LRmix-Studio

![LRmix-Studio interface](image2)

Same functionality as LRmix in a user-friendly GUI!

Lab Retriever

![Lab Retriever interface](image3)

http://www.scieg.org/
Semi-continuous methods

- Use a Pr(DO) and LRs
- Speed of analysis – “relatively fast”
- The methods do not make full use of data - only the alleles present.

What should we do with data below our Stochastic Threshold?

- Continue to use RMNE (CPI, CPE) (not optimal)
- Use the Binary LR with 2p (not optimal)
- Semi-continuous methods with a LR (Drop models)
- Fully continuous methods with LR
Continuous Models

- Mathematical modeling of “molecular biology” of the profile (mix ratio, PHR (Hb), stutter, etc...) to find optimal genotypes, giving WEIGHT to the results.

<table>
<thead>
<tr>
<th>Probable Genotypes</th>
<th>Ac - 40%</th>
<th>Bc - 25%</th>
<th>Cc - 20%</th>
<th>Cq - 15%</th>
</tr>
</thead>
</table>

Some Continuous Model Examples

- TrueAllele (Cybergenetics)
- STRmix (ESR [NZ] and Australian collaboration)
- DNA-View Mixture Solution (Charles Brenner)
- DNAmixtures (Graversen 2013a,b) – open source, but requires HUGIN.

Weights may be determined by performing simulations of the data (Markov Chain Monte Carlo - MCMC).
Fully continuous methods

- Can model drop-out and provide weights for the LR calculation
- Speed of analysis – can vary
- Attempts to use all of the data

MIX13 Participants from 108 Laboratories
46 states had at least one lab participate

Due to the number of laboratories responding and the federal, state, and local coverage obtained, this MIX13 interlaboratory study can be assumed to provide a reasonable representation of current U.S. forensic DNA lab procedures across the community.
Purpose of MIX13 Cases

<table>
<thead>
<tr>
<th>Challenge provided to study responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
</tr>
<tr>
<td>Case 2</td>
</tr>
<tr>
<td>Case 3</td>
</tr>
<tr>
<td>Case 4</td>
</tr>
<tr>
<td>Case 5</td>
</tr>
</tbody>
</table>

According to German Stain Commission (2009): mixture types: 1 = A, 2 = C, 3 = ?, 4 = B, 5 = ?

Case 05 – Ski Mask (Robbery Evidence)

Complex mixture (>3-person) with # of contributors; inclusion/exclusion issues

Scenario

- Evidence: Ski mask recovered at a bank robbery.

- A number of gang-related robberies have targeted several banks in the city. The robberies have typically involved 2-3 perpetrators. A ski mask was recovered in a trash can one block away from the latest bank robbery and is submitted for DNA testing.

- A confidential informant has implicated two suspects in at least three of the armed robberies. Police have obtained buccal swab references from the two suspects identified from the CI, and another known accomplice of the suspects.
No more than 4 alleles at a locus

- Suggests a 2 person mixture

- Peak Height information does not agree

Note: All samples are unrelated (relative testing, mtDNA, Y-STRs, X-STRs, etc...)
Case 05 – 3 Suspects

<table>
<thead>
<tr>
<th>Individual</th>
<th>Suspect 5A</th>
<th>Included</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Suspect 5B</td>
<td>Included</td>
</tr>
<tr>
<td></td>
<td>Suspect 5C</td>
<td>Not in the mixture</td>
</tr>
</tbody>
</table>

**MIX13 Case 5 Outcomes with Suspect C**
(whose genotypes were not present in the mixture)

<table>
<thead>
<tr>
<th># Labs</th>
<th>Report Conclusions</th>
<th>Reasoning points</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>7</strong></td>
<td>Exclude Suspect C</td>
<td>Detailed genotype checks (ID+); TrueAllele negative LR (ID+); assumed major/minor and suspects did not fit (ID+); 4 of 18 labs noted Penta E missing allele 15 (PP16HS)</td>
</tr>
<tr>
<td><strong>3</strong></td>
<td>Inconclusive with C only (A &amp; B included)</td>
<td>All these labs used PP16HS</td>
</tr>
<tr>
<td><strong>22</strong></td>
<td>Inconclusive for A, B, and C</td>
<td></td>
</tr>
<tr>
<td><strong>76</strong></td>
<td>Include &amp; provide CPI statistics</td>
<td>All over the road...</td>
</tr>
</tbody>
</table>

**Range of CPI stats for Caucasian population:**
FBI allele frequencies: **1 in 9** (Labs 12 & 54), **to 1 in 344,000** (Lab 107)

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Case 05

“Couldn’t help but note the need for mix deconvolution software tools for case 05”
(a) Deconvolution as 3p mixture
| [19, 23] | [17, 19] | [18, 20] | 0.0660329755145108E-6 |
| [17, 18] | [17, 19] | [18, 20] | 0.0680006358988452E-6 |
| [16, 18] | [17, 19] | [18, 20] | 2.941235929578498E-6 |
| [18, 20] | [17, 19] | [18, 20] | 2.841235929578498E-6 |
| [17, 20] | [17, 19] | [18, 20] | 0.00245704425394938E-6 |
| [16, 20] | [17, 19] | [18, 20] | 0.0786600202985663E-6 |
| [17, 17] | [17, 18] | [18, 20] | 5.666451887687596E-6 |
| [17, 18] | [17, 19] | [18, 20] | 0.003350163323951E-6 |
| [17, 19] | [17, 18] | [18, 20] | 2.420217178250712E-6 |
| [16, 19] | [17, 18] | [18, 20] | 8.857031348467576E-4 |
| [18, 18] | [17, 20] | [18, 20] | 4.246434606650707E-4 |
| [18, 19] | [17, 20] | [18, 20] | 3.033362533636006E-4 |
| [17, 19] | [17, 20] | [18, 20] | 2.336630681026864E-4 |
| [16, 18] | [17, 20] | [18, 20] | 1.503632946496572E-5 |
| [15, 18] | [17, 20] | [18, 20] | 1.000337645028448E-5 |
| [20, 23] | [17, 18] | [18, 20] | 0.016664914233635E-3 |
| [19, 20] | [17, 18] | [18, 20] | 3.238594037548309E-6 |
| [18, 20] | [17, 18] | [18, 20] | 1.000000070377680E-5 |
| [19, 19] | [17, 18] | [18, 20] | 0.0001553313416210273E-8 |
| [19, 20] | [18, 20] | [18, 20] | 0.0740143012767023E-5 |
| [19, 20] | [18, 20] | [18, 20] | 5.572142414676195E-6 |
| [20, 23] | [17, 18] | [18, 20] | 5.172506487475795E-5 |
| [17, 20] | [17, 18] | [18, 20] | 2.332037915164468E-5 |
| [20, 23] | [17, 18] | [18, 20] | 5.847601715040711E-5 |

| [17, 20] | [17, 18] | [18, 20] | 5.501238565407147E-6 |
| [20, 23] | [17, 18] | [18, 20] | 0.00291845464405823E-3 |
| [17, 20] | [17, 18] | [18, 20] | 6.170835850502268E-5 |
| [18, 20] | [17, 18] | [18, 20] | 8.223527334502755E-5 |
| [17, 20] | [17, 18] | [18, 20] | 0.0026137682065911402E-5 |
| [16, 20] | [17, 18] | [18, 20] | 0.0799450267476601E-5 |
| [20, 20] | [17, 18] | [18, 20] | 1.125641962677777E-5 |
| [21, 24] | [17, 18] | [18, 20] | 3.129162478123258E-5 |
| [17, 20] | [17, 18] | [18, 20] | 2.137546751261907E-4 |
| [17, 17] | [20, 20] | [18, 20] | 3.533444030534655E-5 |
| [16, 18] | [17, 17] | [20, 20] | 1.050946748222445E-5 |
| [16, 18] | [17, 17] | [20, 20] | 4.69399030139292E-6 |
| [18, 18] | [17, 17] | [20, 20] | 1.419229433442988E-5 |
| [20, 20] | [17, 17] | [20, 20] | 0.01744550832575917E-5 |
| [19, 20] | [17, 17] | [20, 20] | 0.0526560406102853E-5 |
| [20, 20] | [17, 17] | [20, 20] | 5.626005837432716E-6 |
| [20, 20] | [17, 17] | [20, 20] | 0.0760276735147763E-5 |
| [17, 20] | [17, 17] | [20, 20] | 0.0022137186731292E-4 |
| [18, 18] | [17, 17] | [20, 20] | 0.01066731919778608E-5 |
| [17, 20] | [17, 17] | [20, 20] | 5.546567555347558E-6 |
| [17, 17] | [18, 20] | [20, 20] | 0.076955254890977E-5 |
| [17, 17] | [17, 20] | [20, 20] | 1.3617100026202756E-4 |
| [16, 20] | [18, 20] | [20, 20] | 2.654021561538798E-5 |
| [17, 20] | [18, 20] | [20, 20] | 5.025324561135745E-6 |

93 Possible Genotype Combinations
Summary

- Probabilistic Methods make better use of the data than RMNE or the binary LR with 2p.

- The goal of the software programs should not be to simply “get bigger numbers” but to understand the details of these approaches and not treat the software as a “black box.”

Summary of Issues

- Use of CPI has significant limitations when it comes to complex mixtures because this approach delivers information regarding the presence of alleles rather than specific suspect genotypes.

- A CPI approach has the potential to falsely include innocent suspects as demonstrated in MIX13 Case 5.

- The U.S. forensic DNA community adopted CPI for simplicity in 1990s and early 2000s when 2-person mixtures were common and have now inappropriately extrapolated the approach to more complex mixtures.
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